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Rheological properties of seed galactomannan from *Cassia nodosa* buch.-hem.

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The solution properties of the galactomannan polysaccharide isolated from the seed of *Cassia nodosa* have been determined for a wide range of concentrations. The gum used has a mannose-to-galactose ratio of about 3·5, $\overline{M_w} = 7{\cdot}01 \times 10^5$ and an intrinsic viscosity of 1210 ml/g.

The transition from dilute to semi-dilute regime, determined from viscometry, occurs at a critical concentration $C_c^* \sim 2{\cdot}5 \pm 0{\cdot}5/[\eta]$ which is similar to the values obtained for some galactomannans and other polysaccharides although it is higher than the value obtained for more rigid polysaccharides. The slope of the log-log plot of specific viscosity versus C at zero shear rate is 6·5 in the more concentrated regime.

These rheological studies clearly reveal the characteristics of a coil in dilute solutions. However, the large dependence of the viscosity on the coil overlap parameter is probably due to interactions because of low galactose content. The concentrated domain is characterized for polymer concentrations higher than 15 g/l where the rheological behaviour reached that of a pseudonetwork (weak gel-like behaviour).

INTRODUCTION

Seed galactomannans, commonly known as seed gums, are important industrial products used in various industries like paper, textile, food, cosmetics, pharmaceuticals, paints, petroleum, etc. They are found as water soluble polysaccharides in seed endosperms of many legumes and consist of a β-(1 → 4)-mannan backbone having single α-(1 → 6)-galactose side chains. They differ from each other in galactose-mannose ratios, molecular weight and fine structure regarding the distribution of the galactose side groups on the main chain.

Many papers have been reported on the rheology of guar and carob gums (Doublier & Launay, 1981; Robinson *et al.*, 1982; Richardson & Ross-Murphy, 1987) and other seed gums (Sharman *et al.*, 1978; Ganter *et al.*, 1992); on the pseudoplastic properties characterized by the power law (Elfak *et al.*, 1979; Whitcomb *et al.*, 1980); and on the solution properties of galactomannans having varying galactose-mannose ratio and molecular weight.

The studies on the effect of concentration and molecular weight on dilute solution behaviour and the dynamic properties of more concentrated solutions clearly revealed the random coil nature of guar galactomannan and the transition from dilute to semi-dilute regimes above a critical polymer overlap concentration. It showed a tendency to form specific polymer-polymer interactions in more concentrated solutions, as evidenced by the high exponent relating specific viscosity to polymer concentration, in contrast to other polysaccharides (Morris *et al.*, 1981).

The present communication deals with the studies of solution properties of galactomannan from the seeds of *Cassia nodosa* buch.-hem. tree (Fam.: *Luguminoseae*; Sub-fam.: *Ceasalpinideae*). It is found in abundance throughout India, known locally as pink cassia, pink mohur or pink and white shower. It can be grown easily in alkaline lands without much care or investment. It is

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a potential source of seed gum, having about 50% endosperm, with nearly 32 weight percentage of polysaccharide on seed basis.

MATERIAL AND METHODS

The seed of *Cassia nodosa* are medium size (10 seeds weight approximately 1·33 g) and having 53·5% endosperm. The endosperm of the seeds was separated by a wet milling process, extracted with methanol– chloroform–water (12:5:3) in a soxhlet, dried at room temperature and at 45°C (yield 50·2%). The polysaccharide was isolated from the powdered endosperm (50 mesh) by extraction with hot water and precipitation with ethanol according to a previous described method (Kapoor *et al.*, 1989), yield 31·7% on seed basis. The polysaccharide was dissolved in water (3% w/v) at 45°C, with continuous stirring for 8 h, and precipitated with saturated barium hydroxide solution. The resulting barium complex was separated by centrifugation and solubilized in 2 M acetic acid with vigorous stirring overnight. The polysaccharide was reprecipitated from the resulting centrifuged solution with ethanol and successively washed with 60, 70, 80, 90 and 95% ethanol. The gum was again dissolved in water (1 g/l), dialysed in distilled water for 48 h, filtered through a 3·0-μm millipore filter, concentrated, precipitated with ethanol and dried under vacuum at 45°C for 48 h, yielding 9·8% on seed basis.

The total carbohydrate concentration was assayed by the phenol–sulphuric acid method (Dubois *et al.*, 1956). The ratio of galactose and mannose was determined after total acid hydrolysis (2 M H_2SO_4 for 15 h) by HPLC for neutral sugars and by GLC of the corresponding alditol acetate derivatives using a Supelco SP 2380 (30 m × 0·53 mm) column with a temperature programme from 195°C (4 min) to 225°C at a rate of 2·5°C/min. GC-MS was performed on a Nermag R-1010C spectrometer equipped with a Delsi Chromatograph model DI-700 and a Digital Equipment Corporation PDP-1173 computer using a SP 2380 (25 m × 0·32 mm) column with a temperature programme of 180–220°C at a rate of 3°C/min.

NMR spectra were obtained on either an AM 300 or AM 400 Bruker spectrometer, both equipped with a process controller, an Aspect 3000 Computer and a variable temperature system. For ^{13}C-NMR, the galactomannan sample was dissolved in D_2O (20 mg/ml) at 80°C. After complete dissolution, the solution was sonicated for 10 min, centrifuged and transferred to a 5-mm tube. ^{13}C-NMR spectra were performed at 80°C under conditions of inverse gated decoupling (proton decoupled spectrum without nOe). Assignments were made with reference to results already published (Noble & Taravel, 1987) and peak integration was performed using the Bruker software. For ^{1}H-NMR, the galactomannan sample was first exchanged in D_2O by repeated evaporations of 1 mg/ml solution and finally dissolved in high quality D_2O (99·96% D). ^{1}H-spectra were obtained at 80°C in the conditions of quantitative analysis.

Steric exclusion chromatography (SEC) was achieved using the multidetection equipment previously described (Tinland *et al.*, 1988). The eluent was 0·1 M NH_4NO_3 with 0·5 g/l NaN_3 as a preservative. The concentration of gum solutions for SEC was 1 g/l. Two Shodex columns, OH-pak 804 and 805, were used in series. The dn/dc was determined in the same solvent with a Brice–Phoenix differential refractometer using a red filter, and was determined to be 0·150 ml/g.

The solutions, 1 g/l, were filtered on Sartorius membranes before injection on the chromatograph; different porosities were used successively from 8 to 0·2 μm. The light scattering detector is a Dawn multiangle, model F, laser photometer ($\lambda = 632{\cdot}8$ nm) equipped with the ambient flow cell aqueous solution, K5. Data were processed using Astra 1–1 software (Wyatt Technology Corporation, USA). Normalisation was achieved from a NaPSS standard ($M_w = 19\,300$, $M_w/M_n < 1{\cdot}10$).

The polymeric solutions at different concentrations were prepared by mixing weighed amounts of the polysaccharide in the necessary quantity of water by continuous stirring for 10–20 h at 25°C.

Viscosity measurements were carried out on a Fica capillary viscometer (0·46 mm diameter) for intrinsic viscosity $[\eta]$ determination. In the other cases the measurements were carried out with a Contraves Low Shear 30 ($10^{-2} < \dot{\gamma} < 128\ s^{-1}$) and a Carri-Med CS50 rheometer equipped with a Rheo 1000 C System and a 5·0 software. The different cones used on the Carri-Med CS50 had diameters of 2, 4 and 6 cm, (cone angle 4°) and a 6 cm diameter with a cone angle of 1°. The shear stress and shear rate can be varied depending upon the geometry used and experimental conditions. All experiments were performed at 25°C.

RESULTS AND DISCUSSION

Polysaccharide characterization

The purified galactomannan was found to be homogeneous. Complete hydrolysis of the polysaccharide showed the presence of D-galactose and D-mannose. The ratios of the constitutive sugars were determined by HPLC and GLC. Sugar characterization was performed with GC-MS; the results are reported in Table 1. During ^{13}C-NMR studies of the galactomannan, the resonance of all carbon atoms were fully resolved and readily identified. The galactose–mannose ratio, calculated from the corresponding peak area, was in good agree-

Table 1. Composition of neutral sugars in *Cassia nodosa* seed galactomannan

Sugar	Method		
	GLC[a]	HPLC	^{13}C-NMR
D-galactose	1·00	1·00	1·00
D-mannose	3·55	3·58	3·46

[a] As alditol acetate derivatives: characteristic fragments have identical m/z in accordance with literature values (Gupta & Grasdalen, 1988).

ment with that obtained by chemical analysis (Table 1). These results are also in good agreement with the previous report (Rizvi *et al.*, 1971), according to which the galactose–mannose ratio of *Cassia nodusa* seed galactomannan is 1:3·5. The polysaccharide was already described as a linear β-(1 → 4) linked D-mannose main chain, with, on the average of every seven mannose units, two side chains of single α-(1 → 6) linked D-galactose. The weight-average molecular weight $\overline{M}_w$ has been found to be 7·01 × 10^5 whereas the number-average molecular weight $\overline{M}_n$ was 4·73 × 10^5. The polydispersity values of the polysaccharide, i.e. $\overline{M}_w/\overline{M}_n$ and $\overline{M}_z/\overline{M}_n$, were 1·48 and 2·27, respectively. The intrinsic viscosity of the galactomannan was 1210 ml/g with a Huggins constant $k' = 0{\cdot}55$. The value of the intrinsic viscosity agrees with the value obtained for the molar mass.

Flow behaviour

The galactomannan solutions having a wide range of polymer concentration from 0·2 g/l to 50 g/l have been subjected to flow behaviour using two viscometers. Viscosities in the Newtonian plateau and their dependence on shear rate were determined. The curves are given in Fig. 1. In the case of lower concentrations, no shear rate viscosity dependence was experimentally obtained in the range of shear stress used for this investigation, but the behaviour becomes shear-thinning for higher concentrations ($C > 2$ g/l). As expected, the shear rate values corresponding to the onset of the viscoelastic behaviour ($\dot{\gamma}_c$) decreased with increasing polymer concentration. From the departure from linearity of the reduced viscosity versus polymer concentrations, we determine a critical concentration for polymer interactions, C^*_c, which must be in the range of the overlap concentration C^*. The experimental value is $C^*_c \simeq 2$ g/l corresponding $C^*_c[\eta] = 2{\cdot}5 \pm 0{\cdot}5$. We notice in Fig. 1 that the viscoelastic behaviour is observed for concentrations higher than C^*_c. The determination proposed for C^*_c gives values in agreement with results obtained for most randomly coiled hydrocolloids

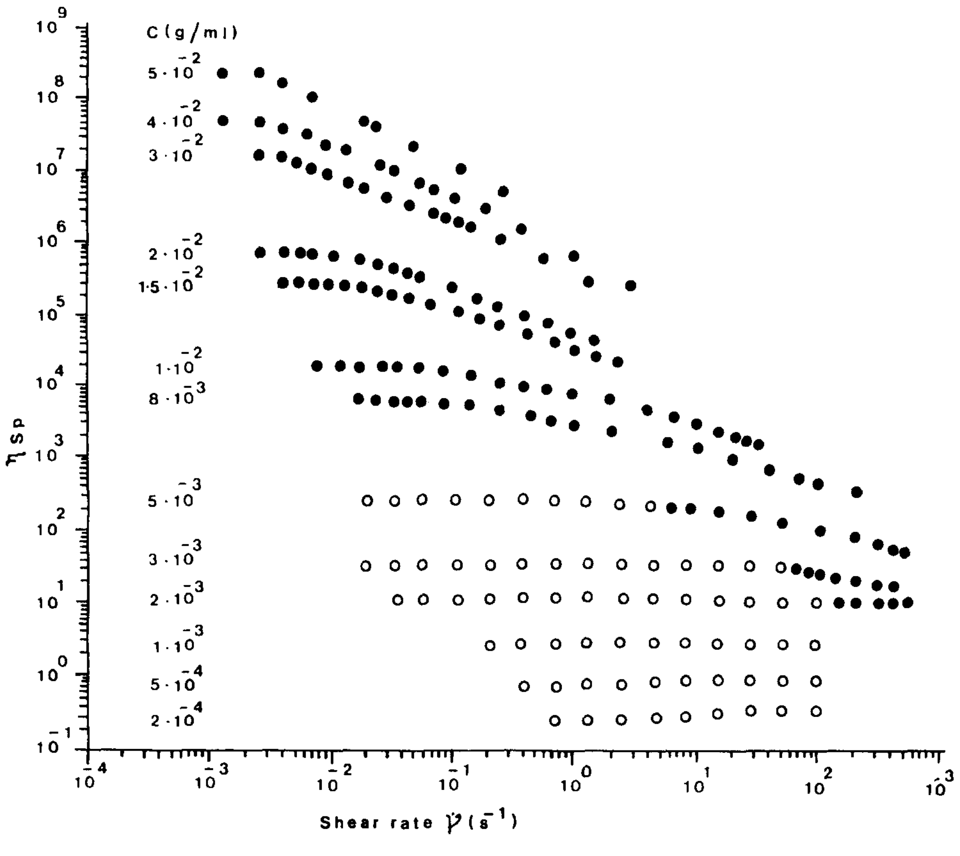

Fig. 1. Variation of the specific viscosity as a function of the shear rate for different galactomannan concentrations in water at 25°C: (○) Contraves Low Shear Viscometer; (●) Carri-Med rheometer.

having a critical overlap parameter in the range of 2–4 (Morris *et al.*, 1981; Launay *et al.*, 1986). A $C_c^*[\eta]$ value equal to 2·6 was recently found for the galactomannan isolated from the seeds of *Mimosa scabrella* (Ganter *et al.*, 1992), and an intrinsic viscosity of 900 ml/g. The lower values found for $C_c^*[\eta]$ for some polysaccharides, e.g. xanthan (Milas *et al.*, 1990), welan (Campana *et al.*, 1990) or succinoglycan (Gravanis *et al.*, 1990), can be attributed to their higher stiffness, reflected by their persistence length (Lp > 30 nm), whereas other polysaccharides described (Morris *et al.*, 1981; Launay *et al.*, 1986; Fouissac *et al.*, 1993) and galactomannans are known to have a persistence length lower than 10 nm (Robinson *et al.*, 1982).

The log–log plot of the concentration dependence of the specific viscosity at zero shear rate (η_{sp_0}) is shown in Fig. 2. On the same figure the variation of log η_{sp} is given as a function of log $C[\eta]$, with $C[\eta]$ the overlap parameter. This curve corresponds to the relationship:

$$\eta_{sp0} = C[\eta] + k'(C[\eta])^2 + B(C[\eta])^n \qquad (1)$$

which allows polymers with different molar masses, i.e. different intrinsic viscosities to be compared.

For higher values, the logarithmic dependence of η_{sp} versus C becomes linear, with a slope of 6·5. Consequently, the following equation is obtained for higher polymer concentrations:

$$\eta_{sp_0} \sim (C[\eta])^{6\cdot 5} \sim C^{6\cdot 5}M^{4\cdot 7} \text{ or } {\sim}C^{6\cdot 5}M^{6\cdot 37}. \qquad (2)$$

In this equation the possible values of the Mark Houwink parameter, $a = 0{\cdot}723$ (Robinson *et al.*, 1982) or 0·98 (Doublier & Launay, 1981), are taken into consideration. The exponents are higher than those usually found for coils in good solvent, but are in good agreement with those found by Doublier & Launay (1981) for galactomannan with a mannose–galactose (M/G) ratio of 4·0 and $\eta_{sp_0} \sim C^{6\cdot 65}$ (Table 2). The high values of the specific viscosities and their dependence upon the polymer concentration seem to be a characteristic of galactomannans with a low galactose content.

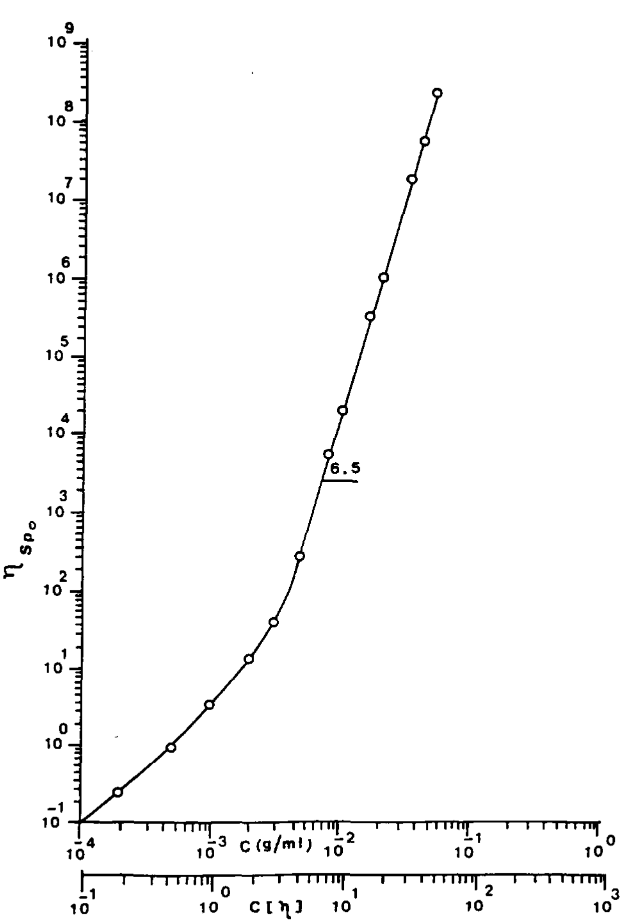

Fig. 2. Dependence of the logarithm of the specific viscosity in the Newtonian plateau η_{sp_0} as a function of the logarithm of the galactomannan concentration, and the overlap parameter $C[\eta]$.

The galactomannan solutions show viscoelastic behaviour above a critical shear rate and polymer concentration. The relationship between the viscosity and the shear rate can be expressed by $\eta \sim \dot{\gamma}^n$. The values of the slope n as a function of the overlap parameter $C[\eta]$ are presented in Fig. 3. The limiting slope is given in Table 2 along with different characteristics obtained which are compared to results previously reported for galactomannans with varying contents of galactose and mannose. No direct correlation seems to exist between n and the galactose content. However, a conclusion is difficult to make, as different ranges of $C[\eta]$ probably

Table 2. Specific viscosity at $C[\eta]$ = 10; slope S of log η_{sp_0} versus log $C[\eta]$ for the highest $C[\eta]$values; limiting slope, n, of viscosity versus shear rate in the viscoelastic domain and galactose–mannose ratio of different galactomannans

	Reference				
	a	*b*	*c*	*d*	*e*
η_{sp_0} at $C[\eta] = 10$	5500	6300	2200	300	600
S	6·4	6·65	4·5	4·2	5·1
n	−0·81	−0·73	−0·7	−0·65	−0·79
M/G	3·55	4·00	2·00	1·1	1·56

a: Present work; *b*, *c*: from Doublier & Launay (1981) for locust bean and guar samples; *d*: from Ganter *et al.* (1992) for galactomannan from *Mimosa scabrella* seeds; *e*: Robinson *et al.* (1982) for guar samples.

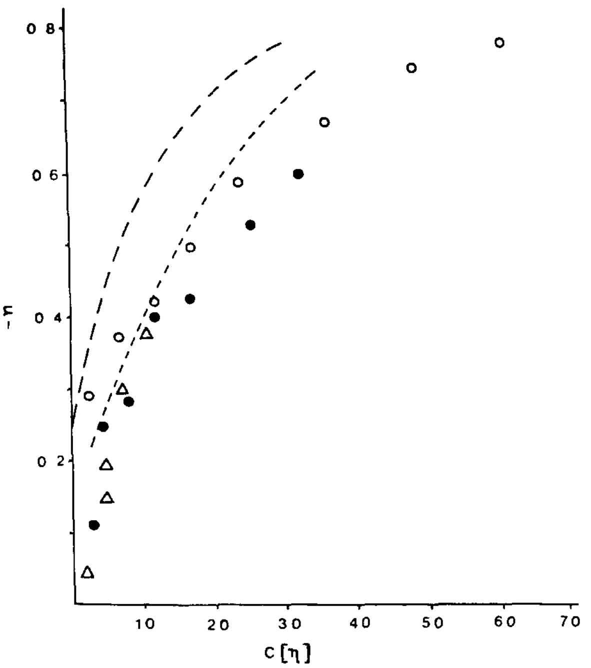

Fig. 3. Limiting slope, n, of log η versus log $\dot{\gamma}$ in the viscoelastic domain, as a function of the overlap parameter $C[\eta]$ for *Cassia nodosa* galactomannan (○). Comparison with galactomannans from other sources: *Mimosa scabrella* (●; Ganter *et al.*, 1992); carob and lucerne (△, Sharman *et al.*, 1978); xanthan (— — —, Milas *et al.*, 1990); and polystyrene in toluene (- - - - -, Graessley, 1974).

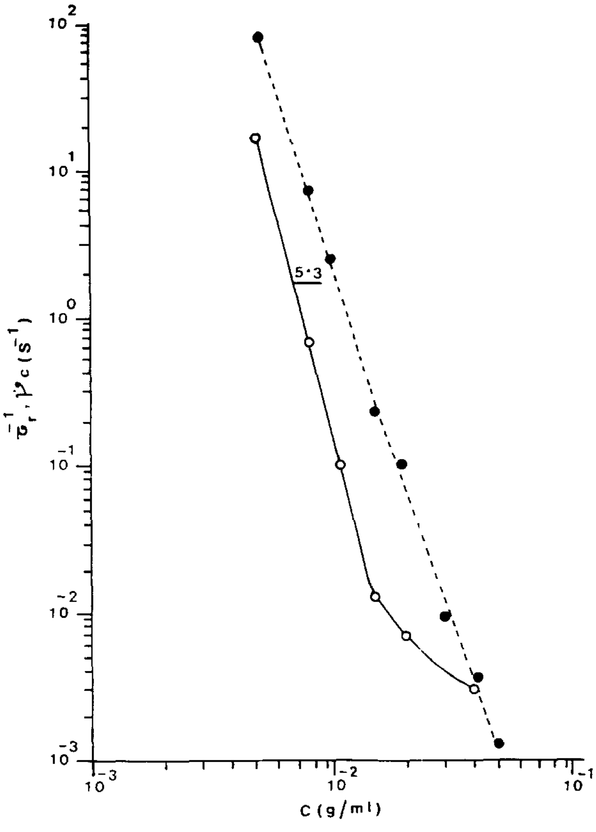

Fig. 4. Critical value of the shear rate ($\dot{\gamma}_c$) for the onset of viscoelastic behaviour (○) as a function of polymer concentration. Comparison with τ_r^{-1} values (●) from eqn (3).

have been used for determining n and no information is given concerning the polydispersity of the various samples.

In Fig. 4, the critical value of shear rate ($\dot{\gamma}_c$) for the onset of viscoelastic behaviour is plotted as a function of polymer concentration. Three domains characterize this relationship. Below a concentration of about 2 g/l ($C[\eta] \sim 2{\cdot}5 \pm 0{\cdot}5$) or below C_c^*, $\dot{\gamma}_c$ cannot be determined from the rheometers used in this work. Its values should be higher than 10^3/s. This corresponds to the dilute regime where the Huggins law applies. Between 2 g/l and about 15 g/l, $\dot{\gamma}_c$ decreases strongly when the polymer concentration increases. In a log–log representation, the variation is linear with a slope equal to about $-5{\cdot}3$. This domain corresponds to the semi-dilute regime. It has been proposed in this regime to relate $\dot{\gamma}_c$ to τ_r, the longest relaxation time in the solution (Graessley, 1974; Ferry, 1980). Thus $\dot{\gamma}_c \sim \tau_r^{-1}$ and

$$\tau_r = 6(\eta_0 - \eta_s)\frac{M}{\pi^2 CRT}, \qquad (3)$$

where η_0 and η_s are the Newtonian viscosity of the polymeric solution and the solvent viscosity, respectively. As we have shown previously, $\eta_0 \sim C^{6{\cdot}5}$, and we find from eqn (3) that $\tau_r^{-1} \sim C^{-5{\cdot}5}$, which corresponds well to the experimental variation of $\dot{\gamma}_c$ ($\dot{\gamma}_c \sim C^{-5{\cdot}3}$). However, the theoretical τ_r^{-1} values calculated from eqn (3) are higher than $\dot{\gamma}_c$ (Fig. 4), while for galactomannan with a lower mannose–galactose ratio these values are similar (Ganter *et al.*, 1992). The polydispersity and the presence of aggregates may explain this difference. The viscoelastic behaviour appears at lower $\dot{\gamma}_c$ values for galactomannans with low galactose content. Above $C \sim 15$ g/l, $\dot{\gamma}_c$ seems to become less dependent on the concentration ($\dot{\gamma}_c \sim C^{-1{\cdot}5}$). This behaviour can be related to that obtained on the diffusion coefficient in xanthan solutions (Tinland *et al.*, 1990) in which a critical concentration (C^{**}) is introduced for the entrance in a concentrated regime related to the semi-flexible character of this polymer. Theoretically, the onset of this regime is predicted to appear at $C^{**} \sim 21$ g/l, using a wormlike chain model (Tinland *et al.*, 1990) with a persistence length of 70 Å and a mass-per-unit length equal to 42 g/Å. The persistence length value is obtained from the Yamakawa & Fujii (1974) theory, which applies to wormlike polymer using $[\eta]$ and M_w values measured on the present sample. The value obtained is compatible with the Mark Houwink parameter ($a \sim 0{\cdot}72$–$0{\cdot}98$) given in the literature. The C^{**} value is in the range of that obtained from the change in log $\dot{\gamma}_c$ versus log C relationship. In this regime, t_r^{-1} (eqn 3) no longer represents the $\dot{\gamma}_c$ variation with polymer concentration. Nevertheless, flow experiments, at lower shear rate, or creep experiments are

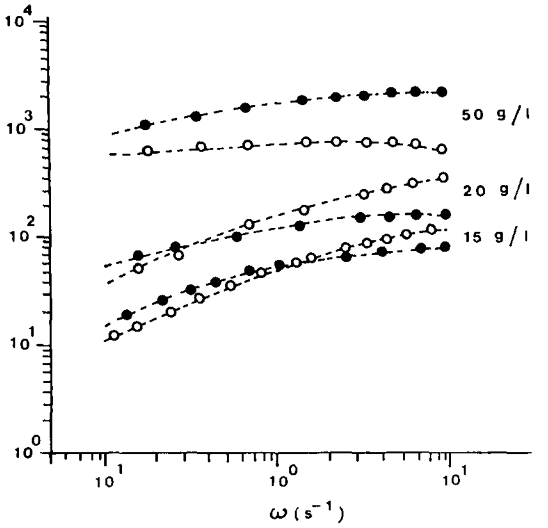

Fig. 5. $G'(\omega)$ (●) and $G''(\omega)$ (○) moduli as a function of the frequency ω, for different galactomannan concentrations in water at 25°C.

needed to confirm this change, which has been recently obtained with hyaluronate solutions (Berriaud *et al.*, 1994).

Dynamic measurements

At concentrations lower than about 10 g/l the viscosities of galactomannan solutions are too low to obtain reliable values for G'' and G', the loss and elastic modulus, respectively. Figure 5 gives the results obtained for polymer concentrations of 15, 20 and 50 g/l. At 50 g/l, G' reaches a plateau for a value equal to about 4×10^3 Pa. From this value a mass between entanglements can be estimated at this polymer concentration $M_e = CRT/G'$. The value obtained ($M_e = 31\,000$) corresponds to the formation of a pseudonetwork.

CONCLUSION

The galactomannan from the seeds of *Cassia nodosa* is characterized by its low galactose content. This contributes to some specific rheological behaviours even if the boundaries between the different regimes are not affected. The main effects correspond to a large dependence of the viscosity and the critical shear rate ($\dot{\gamma}_c$), over which the viscoelastic behaviour takes place, upon the polymer concentration especially in the semi-dilute regime. For similar intrinsic viscosities, solutions of galactomannans with low galactose content exhibit higher viscosities and lower $\dot{\gamma}_c$ values compared to samples with a high galactose content.

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